Mineral Metabolism Factors Predict Accelerated Progression of Common Carotid Intima-Media Thickness in Chronic Kidney Disease. The NEFRONA Study.

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Abstract

Background. The leading cause of premature death in chronic kidney disease (CKD) is cardiovascular disease (CVD), but risk assessment in renal patients is still challenging. The aim of the study is to analyze factors predicting accelerated progression of common carotid intima-media thickness (CCIMT) in a CKD cohort after two years of follow-up (2010-2012)**.**

Methods. The study included 1152 patients from the NEFRONA cohort with CKD stage 3 to 5D and without clinical history of previous CVD. CCIMT was measured at the far wall on both common carotids. CCIMT progression was defined as the change between CCIMT at baseline and CCIMT at 24 months for each side, averaged and normalized as change per year. Accelerated progressors were defined as those with a CCIMT change over the 75th percentile.

Results. The median CCIMT progression rate was 0.0125 mm per year, without significant differences between CKD stages. The cut-off value for defining accelerated progression was 0.0425 mm per year. After adjustment, age was a common factor among all CKD stages. Traditional cardiovascular risk factors like diabetes and systolic blood pressure were predictors of progression in CKD stages 4-5, whereas HDL and LDL cholesterols predicted progression in women in stage 3. Mineral metabolism factors predicting accelerated progression were serum phosphorus in stage 3 and 5D; low 25-hydroxyvitamin D and parathyroid hormone levels over 110 pg/ml, in stage 4-5; and iPTH levels out of the recommended range in stage 5D.

Conclusions. Mineral metabolism parameters might predict accelerated CCIMT progression from early CKD stages.

Keywords: intima-media thickness, chronic kidney disease, atheromatosis progression, parathyroid hormone, phosphorus, vitamin D.

INTRODUCTION

Atheromatosis is a multifactorial inflammatory fibroproliferative process of the artery wall, with a high prevalence in chronic kidney disease (CKD) and one of the most common forces behind cardiovascular disease (CVD) [1,2]. Individuals with chronic kidney disease are at a higher risk of cardiovascular events than the general population [3], but risk assessment in this population is difficult due to the lack of accuracy of current algorithms [4]. Nonetheless, the use of non-invasive vascular ultrasound imaging is an emerging tool with promising outcomes for cardiovascular risk assessment in CKD patients [5,6].

Carotid intima-media thickness (IMT), defined as the distance between the intimal-luminal and the medial-adventitial interfaces of the artery wall, is a reliable surrogate marker with predictive value on cardiovascular events in the general [7] and CKD populations [6,8]. In early stages of atheromatosis, IMT increases before the development of the plaque and it has been shown that individuals in which IMT progresses faster would be at a higher risk for future events [9].

CKD is a multifactorial complex disease where a wide range of metabolic alterations are involved. Besides traditional cardiovascular risk factors, specific renal factors can play an important role in the early onset of CVD, explaining the excess risk attributable to kidney disease [10]. One of the main complications secondary to CKD is the dysregulation of mineral metabolism parameters, namely CKD related mineral-bone disorders (MBD) [11]. Disorders in the secretion of parathyroid and vitamin D hormones, main regulators of calcium and phosphorus homeostasis, have an important effect on many organs and tissues, including the vessels [12]. These alterations have been associated with an increased risk of cardiovascular events in dialysis patients [13,14]. Nevertheless, CKD-MBD begin early in the course of CKD and little is known about its predictive value in subclinical atheromatosis progression [15]. Studies examining the relationship between CKD and IMT commonly focus on analyzing cardiovascular risk factors associated with an increased IMT in a cross-sectional nature [16,17], and there are scarce data about IMT progression in this specific population [9,15,18]. Identification of the specific factors that influence IMT progression in CKD could uncover new therapeutic strategies to decrease

cardiovascular morbidity and mortality. Therefore, the aim of the present study was to analyze the factors associated with an accelerated progression of the common carotid artery intima-media thickness (CCIMT) in 1152 patients with stage 3 to 5D CKD, after two years of follow-up.

MATERIALS AND METHODS

Study Population

NEFRONA is an observational multicenter prospective CKD cohorts study designed to evaluate the subclinical atherosclerosis burden and the predictive value of biomarkers and surrogate measures on CVD. Its design and baseline characteristics have been previously described [19,20]. Briefly, between 2010 and 2012, the NEFRONA study enrolled 2.445 patients free from clinical CVD aged 18-76 and who were recruited from 81 spanish hospitals and dialysis clinics. From the original NEFRONA Study cohort of 2445 patients with CKD, 888 patients were excluded from the 24-month follow-up visit. Namely, patients who had stenotic carotid plaque or had ankle-brachial index (ABI) <07 (n=118), had a cardiovascular event during the 2 year follow-up (*n*=97), received a renal transplant (*n*=371), died from non-cardiovascular causes (*n*=58) or second visit non-attendees (*n*=244) were excluded from the follow-up visit. Some patients who attended the 24-month ultrasound visit were excluded because of missing CCIMT values (*n*=31*)*, having a plaque located in common carotid artery (CCA) at baseline (*n*=154*);* or incomplete laboratory data (*n*=220*)*. Finally a total of 1552 were included in the present study (figure 1).

All participants were included after providing informed consent. The study was approved by each local hospital ethics committee.

Carotid ultrasound

High-resolution B-mode ultrasound was conducted using a standardized protocol, and following the recommendations of the Mannheim Consensus [21]. CCIMT was measured at the far wall of both common carotid arteries in plaque free segments, 1 cm proximal to the carotid bulb and taking into account the mean value of 150 measurements performed on a 10 mm segment. The presence of atheromatous plaques was defined as an IMT ≥ 1.5 mm protruding into the lumen.

Ultrasound explorations were carried out by the same itinerant team of 5 trained technicians. Images were analyzed in a blinded fashion by a single reader from the UDETMA (Unit for the Detection and Treatment of Atherothrombotic Diseases, Hospital Universitari Arnau de Vilanova, Lleida, Spain). For the examinations, a computer-supported image analysis system (Vivid BT09 and the semi-automatic software EchoPAC Dimension, General Electric) was used.

To assess the quality of the measurements a sample of 20 individuals was measured 3-5 times on different days, obtaining an intraclass correlation coefficient of 0.93.

CCIMT progression was defined as the difference between CCIMT at baseline and CCIMT at 24 months for each side, and then averaged and normalized as change per year. Based on the aim of the study, accelerated CCIMT progression was defined as an IMT change equal or above the 75th percentile.

Clinical and Biochemical data

At recruitment, the itinerant team reviewed the hospital medical records for each selected subject and collected demographic data, medical history of comorbidities and treatments. Anthropometrical data and vital signs were obtained by the itinerant team using standardized methods[22]. Biochemical parameters were obtained from a routine fasting blood test performed not further than 3 months apart (either before or after) from the vascular exploration. Furthermore, in dialysis patients, blood sample was obtained before the second session of the week.

Special attention was paid to those parameters measured with different methods. Particularly, iPTH was corrected using an established conversion method [23]. High-sensitivity C-reactive protein (hsCRP) and 25-hydroxyvitamin D were both analyzed in a centralized laboratory; hsCRP was determined by immunoturbidimetric method (Roche/Hitachi modular analytics) and 25-hydroxyvitamin D levels by Elisa (IDS, UK).

iPTH concentrations were categorized according to KDOQI guidelines for each renal stage [24], comparing the extreme groups with the target levels.

The MDRD4 equation was used to estimate GFR, defining three CKD stages; stage 3 GFR 60-30 ml/min per 1.73 m^2; stage 4-5 GFR<30 ml/min per 1.73 m^2; or dialysis.

Statistical analysis

All analyses were conducted stratified by CKD stages because many of the potential predictive factors and confounders show a high variation along the CKD spectrum (i.e. phosphate and PTH levels are relatively normal in early stages whereas in late stages those levels increase sharply. On the contrary vitamin D levels are relatively stable until they drop in dialysis patients). Descriptive analysis included absolute and relative frequencies for qualitative variables, compared by Pearson chi-square test; and mean and standard deviation or median and interquartile range for quantitative data, compared by Student's T or Mann-Whitney tests depending on the normality of the distribution. Comparisons of more than two groups of data were performed using one-way Anova (with Bonferroni post-hoc analyses for multiple comparisons) for parametric data or Kruskal-Wallis for non-parametric data.

Factors predicting accelerated CCIMT progression were estimated by fitting a multivariate logistic regression model for each CKD stage. In order to avoid the over-representation of non-accelerated progressors, sampling weights were assigned to each group.

Special attention was paid for nonlinear relationships, evaluated by linear spline models with different knots (i.e. body mass index on dialysis and iPTH in all stages). Highly skewed variables were log-transformed before inference testing in regressions models (i.e hsCRP and 25-hydroxyvitamin D). Explanatory variables strongly correlated were also analyzed, remaining those that best fitted the model (i.e systolic blood pressure versus pulse pressure and low-density lipoprotein cholesterol (LDL) versus total cholesterol).

All multivariate analyses were adjusted for available variables based on the existing cardiovascular risk literature and for specific CKD factors that may play a role on CCIMT progression, according CKD stage.

Models for CKD stage 3 and stages 4-5 were adjusted for age, sex, smoking (current and former versus non-smokers), diabetes, body mass index, systolic blood pressure, LDL and HDL cholesterol, log-transformed high-sensitive C-reactive protein, total calcium, phosphorus, log-

transformed 25-hydroxyvitamin D and ranges of iPTH concentrations (using as reference KDOQI-recommended target range).

Model for CKD 5D was adjusted for age, sex, smoking (current and former versus non-smokers), diabetes, body mass index in tertiles (reference middle tertile), systolic blood pressure, LDL and HDL cholesterol, log-transformed high-sensitive C-reactive protein, albumin, total calcium, phosphorus, log-transformed 25-hydroxyvitamin D, ranges of iPTH concentrations (using as reference KDOQI-recommended target range) and time on dialysis (months).

Specific multiplicative interaction effects between sex and explanatory variables were tested and included in the model only if statistically significant, according to Likelihood Ratio test. The calibration of the models was assessed using Hosmer-Lemeshow χ^2 statistic test. Statistical significance was set at a p-value <0.05. All analyses were performed using the SPSS software (version 21).

RESULTS

Baseline characteristics according to CKD stage

Demographic and clinical data are depicted in table 1. Age and body mass index decreased significantly in more advanced CKD stages. Furthermore, the percentage of men was higher in stage 3 and decreased in more advanced CKD stages.

Traditional cardiovascular risk factors (diabetes, hypertension and dyslipidemia) were common in stage 3 and 4-5, and decreased significantly in stage 5D. Altered mineral metabolism parameters (hyperphosphatemia, hyperparathyroidism, hypovitaminosis D) were more evident in more advanced CKD stages.

Two-Year Progression in CCIMT

CCIMT at baseline was significantly higher in stage 3 than in stage 4-5 or stage 5D. The median CCIMT progression rate was 0.0125 mm per year. There was neither differences in median progression rate between different CKD stages (stage 3: 0.0138 mm, stage 4-5: 0.0100 mm, stage 5D: 0.0225 mm) nor in the 75th percentile, used as the threshold to define accelerated progression (stage 3: 0.0425mm, stage 4-5: 0.0425mm, stage 5D: 0.0400mm). Figure 2~~1~~ shows the mean

values of CCIMT at baseline and the median changes after follow-up, according to sex and CKD stage. Mean CCIMT at baseline was significantly higher in men in stage 3 and 4-5 CKD, whereas the yearly change of median CCIMT was greater among men in dialysis.

Baseline characteristics according to group of progression and CKD stage

The characteristics between groups of accelerated and non-accelerated progressors by CKD stage are given in Table 2. Across all CKD stages, accelerated progressors were on average 4 to 6 years older and had a higher prevalence of carotid plaque at bifurcation or internal carotid segments than non-accelerated progressors. In individuals in stage 3, no significant differences were found between groups in any other of the variables analyzed. In stage 4-5, accelerated progressors were more likely to be diabetics, had higher systolic blood pressure and lower serum 25-hydroxyvitamin D levels than non-accelerated progressors. In stage 5D, accelerated progressors were more frequently men, with greater body mass index, higher serum phosphorus, and most of them were taking vitamin D treatments.

Predictors of accelerated CCIMT progression

Traditional Risk Factors

After adjusting for potential confounders (table 3), age was independently associated with an accelerated progression across all CKD stages.

Diabetes and systolic blood pressure were predictors of accelerated CCIMT progression, among individuals in stage 4-5 and male sex was also a predictor in CKD stage 5D.

Interaction effects were found between low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL) and sex in stage 3. Among women, LDL was positively associated with accelerated progression, whereas HDL was inversely related to accelerated progression (supplementary data figure 1 and table 1).

Non-Traditional Risk Factors

Serum phosphorus was independently associated with accelerated CCIMT progression among individuals in stage 3 and stage 5D, and 25-hydroxyvitamin D was inversely associated with accelerated progression in stage 4-5.

Figure 3 ~~2~~ shows a U-shape relationship between iPTH levels and accelerated progression across all CKD stages, reaching statistical significance in individuals with iPTH levels over 110 pg/ml in stage 4-5, and among those in stage 5D with the serum iPTH lower than 150 pg/ml or above 300 pg/ml.

The potential confounding effect of the treatment with erythropoietin or intravenous iron was tested and disregarded.

DISCUSSION

This longitudinal study shows for the first time a strong and independent relationship between serum phosphorus, 25-hydroxyvitamin D and iPTH levels with accelerated CCIMT progression in individuals with moderate to end-stage renal disease without previous cardiovascular disease. In our study, the CCIMT change cut-off value to define accelerated progression ~~progressors~~ (0.0425 mm/y) is clearly higher than the normal rate in general population (defined in several studies to be between 0.0018 and 0.006 depending on sex)[25–27]. Furthermore, a change of 0.035mm/y has been defined to significantly increase the risk of future cardiovascular events[28]. The annual overall CCIMT change of 0.024 mm is in agreement with other studies in renal patients [9,18] and other high risk populations like type 2 diabetic patients[29]. We did not find differences in CCIMT progression between CKD stages, in contrast with a study conducted by Desbien et al [9] which found an effect of renal function on IMT progression. This discrepancy might be explained by the differences in the cohort, as the NEFRONA cohort is composed of younger individuals, without prior cardiovascular disease. When the median change of progression was stratified by sex, it was significantly higher in men than in women in dialysis patients. In further multivariate analysis, being male was an independent factor predicting an accelerated progression in dialysis. This fact could explain, at least in part, previous results showing an independent and significant effect of male sex on cardiovascular events in dialysis patients [30]. However, whether CCIMT progression can predict future events remains to be determined, as controversial results have been published [9,31].

In the present study, age has been found to be one of the most consistent factors predicting CCIMT progression, as it has been already reported in numerous studies [9,18]. Thus, age remains as the only factor that predicts accelerated progression of IMT across all CKD stages.

In the general population, IMT progression can also be partly explained by the presence of lipid disorders [32]. However, the relationship between serum cholesterol and CVD is more complex in patients with CKD, probably due to the cholesterol-lowering effect of systematic inflammation, which increases as GFR decreases [33]. Indeed, three large randomized clinical trials have failed to show a beneficial effect of lipid-lowering therapy in reducing mortality in dialysis patients despite significant reduction in LDL [34]. In our study, no effect of LDL or HDL cholesterol levels was found in stage 4-5 or in dialysis patients. Even so, in stage 3 higher levels of LDL and lower levels of HDL predicted accelerated atherosclerosis among women. Our findings are similar to previous studies in which dyslipidemia was associated with increased IMT values in the early stages of renal disease[17,35]. Furthermore, the differential effects of lipids on IMT depending on sex have been previously demonstrated[36].

One of the most important results of this study is that specific parameters of mineral metabolism play a key role in the fast development of subclinical atheromatosis. We found that serum phosphorus contributes to the accelerated CCIMT progression in CKD stage 3 and in dialysis. It has been reported that phosphorus load, even in the absence of outright hyperphosphatemia, could be an important promoter of atheromatosis [16,37] and several studies have correlated serum phosphorus levels with increased cardiovascular risk in dialysis patients [13,38].

It is noteworthy that we found an independent association between serum PTH and CCIMT accelerated progression. Although the tendency for a U-shaped curve for iPTH levels is present in all the stages, it only reached statistical significance in dialysis patients and in patients with levels over the recommended target in stage 4-5. The fact that K-DOQI proposed targets are not easily achieved is a common issue that has been previously reported [11]. It has also been documented that both, low and high serum iPTH increase the risk of mineral metabolism disturbances, soft tissue calcifications and cardiovascular mortality in dialysis patients [13,14]. Nonetheless few

stu[39,40]. Blondon et al. evaluated the associations of PTH and 25-hidroxyvitamin D with progression of carotid IMT after 9.4 years of follow-up in 3251subjects from the Multi-Ethnic Study of Atherosclerosis, and they found no evidence of an association between these hormones and progression [39]. Reis et al also failed in finding an association between PTH and carotid IMT in a community-based cohort study of 654 participants[40]. However, both studies were general population-based cohorts with much lower levels of PTH than the ones reached in our CKD population, and that may partly explain the discrepancies with our results. In fact Choi et al. found an association between PTH and carotid IMT among postmenopausal women[41] and a recent study based in a CKD cohort dies include iPTH concentrations when assessing IMT demonstrated a regression of IMT after renal transplantation, showing a risk reduction when parameters as iPTH and phosphorus were corrected [15]. The underlying mechanisms by which iPTH affects IMT progression are complex and multifactorial. Indeed, iPTH receptors have been found in endothelial cells [42], and smooth muscle cells [43]. Thus, on the one hand, a lower iPTH receptor activation in vascular smooth muscle cells could enhance IMT progression by a WNT-mediated pathway [44]. On the other hand, high iPTH levels could indirectly increase IMT by activating local production of active vitamin D, which has also been shown to increase proliferation of the medial layer [45]. In endothelial cells, iPTH may also have dual effects on IMT because it can increase nitric oxide production but also RAGE and IL6 [46].

Finally, the effect of vitamin D is also of interest. A role for low levels of 25-hidroxyvitamin D has been found only in stage 4-5. Although the bivariate analysis showed a similar difference in 25-hidroxyvitamin D levels in dialysis patients, the significance of the effect disappeared in subsequent multivariate analysis. Nevertheless, the fact that vitamin D treatment was associated to CCIMT progression might hide the potential benefit of the effect of 25-hidroxyvitamin D. It has long been known that endothelial and vascular smooth muscle cells also express vitamin D receptors, and that its activation shows potential benefits in cardiovascular health [47]. Moreover, clinical studies have shown a strong association between vitamin D levels and cardiovascular mortality in renal patients [48]. In our study the absence of predictive value of serum 25-

hidroxyvitamin D in stage 5D, may point to a possible lack of effects of native vitamin D supplementation in atherosclerosis progression in dialysis patients.

Although the current study provides relevant data regarding the relationship between a wide range of potential risk factors for atheromatosis and IMT progression, the results should be interpreted within the context of an exploratory research about accelerated CCIMT progression, showing several limitations. Firstly, the short follow-up period presumes small changes in CCIMT and may have prevented us from obtaining associations among other meaningful factors studied. Nevertheless, the assessment of accelerated progression avoids in part this limitation, helping us to understand which factors predict a greater change in IMT. Secondly, the assessment of the CCA could lead us to find factors more related to this proximal area, historically associated with a higher shear stress. However, CCIMT represents a more accurate measurement than others segments of the vessel wall [21,49]. In addition, unmeasured confounding parameters such as fibroblast growth factor 23 levels impede us to make a comprehensive analysis of all the known factors affecting mineral metabolism. Finally, misclassification owed to measurement errors, which is susceptible to random errors and short-term variability, and residual confounding due to categorical transformations, might bias the results toward the null.

Despite of these limitations the present study has several strengths. The NEFRONA cohort has an adequate sample size and centralization of analytical parameters and image readings. The absence of prevalent CVD together with an adjustment for a wide range of potential confounders, which are concomitant and often overlooked, may allow us to identify independent associations towards enhancing the current knowledge of the underlying mechanisms of atherogenesis in CKD.

In summary, the present study demonstrates that independently of traditional cardiovascular risk factors, specifics factors related to CKD appear to be relevant, suggesting a potential role of serum phosphorus, vitamin D and iPTH concentrations in the accelerated subclinical atheromatosis process, which also begins in earlier stages of CKD.

FIGURE LEGENDS

Figure 1. Flow chart of patient selection.

Figure 2 ~~1~~. Mean common intima-media thickness (CCIMT) at baseline and median change in CCIMT (mm/year) stratified by sex and CKD stage. Light gray bars indicate mean CCIMT at baseline in women. Dark gray bars indicate mean CCIMT at baseline in men. Dashed light gray line indicates median change in CCIMT among women. Dashed black line indicates median change in CCIMT among men. Error bars represent the standard error and the median absolute deviation, respectively. Mean CCIMT at baseline was increased in men compared with women in CKD 3 and CKD 4-5 (*:$p < 0.05$). Median change in CCIMT was increased in men compared with women in CKD 5D(*:$p < 0.05$).

Figure 3 2. Frequencies and Odds Ratio of iPTH levels for accelerated CCIMT progression, by CKD stage. Bottom part of the figure shows percentage of accelerated progressors depending on iPTH levels distributed according to KDOQI guidelines (black bars). Top part of the figure shows the adjusted odds ratio (95% CI) for accelerated progression of CCIMT. CKD stage 3 and stages 4-5 were adjusted for age, sex, smoking (current and former versus non-smokers), diabetes, body mass index, systolic blood pressure, LDL and HDL cholesterol, log high-sensitive C-reactive protein, total calcium, phosphorus, log 25-hydroxyvitamin D and ranges of iPTH concentrations (reference: KDOQI recommended target range). CKD 5D was adjusted for age, sex, smoking (current and former versus non-smokers), diabetes, body mass index in tertiles (reference: middle tertile), systolic blood pressure, LDL and HDL cholesterol, log high-sensitive C-reactive protein, albumin, total calcium, phosphorus, log 25-hydroxyvitamin D, ranges of iPTH concentrations (reference: KDOQI recommended target range), albumin and time on dialysis (months).

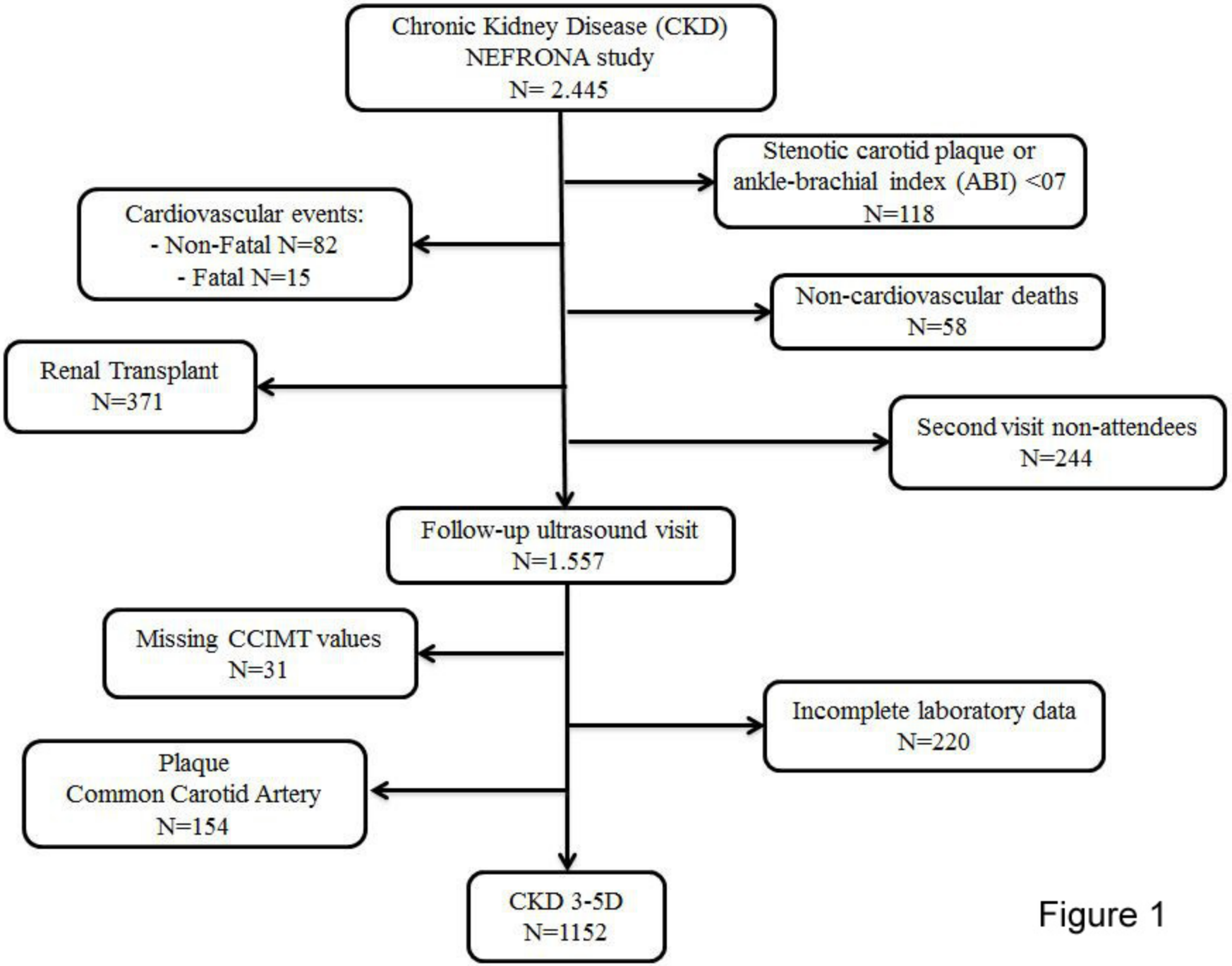

Chronic Kidney Disease (CKD)
NEFRONA study
N= 2.445
Stenotic carotid plaque or ankle-brachial index (ABI) <07
N=118
Cardiovascular events:
- Non-Fatal N=82
- Fatal N=15
Non-cardiovascular deaths
N=58
Renal Transplant
N=371
Second visit non-attendees
N=244
Follow-up ultrasound visit
N=1.557
Missing CCIMT values
N=31
Incomplete laboratory data
N=220
Plaque
Common Carotid Artery
N=154
CKD 3-5D
N=1152

Figure 1

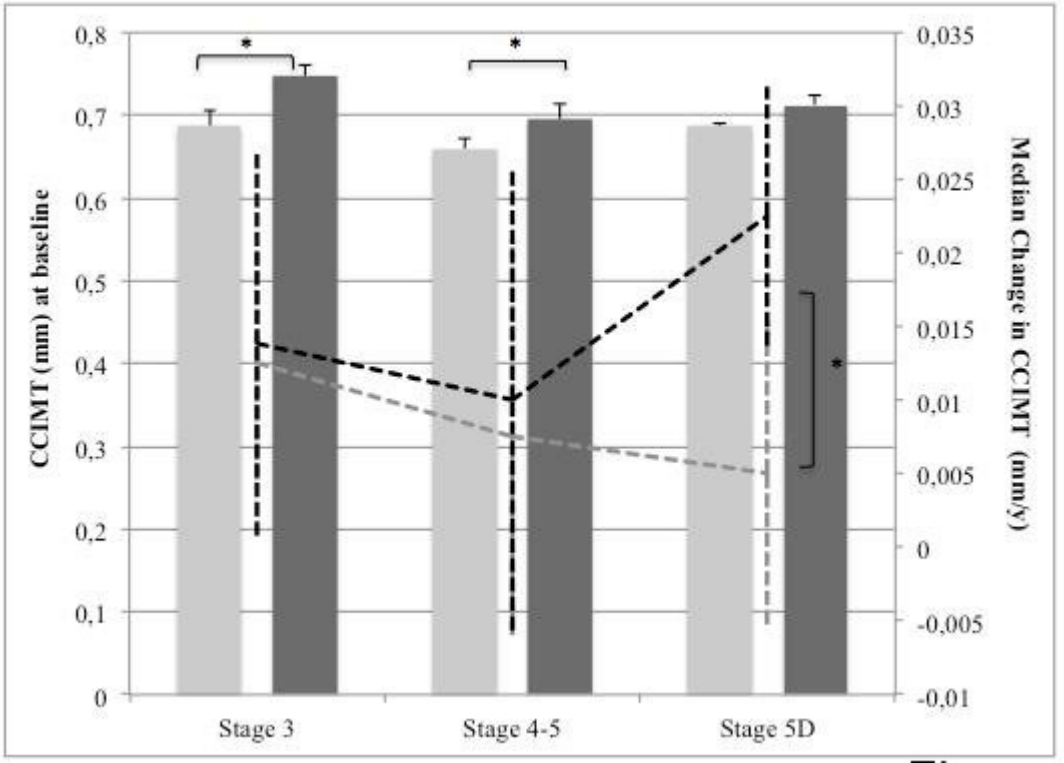
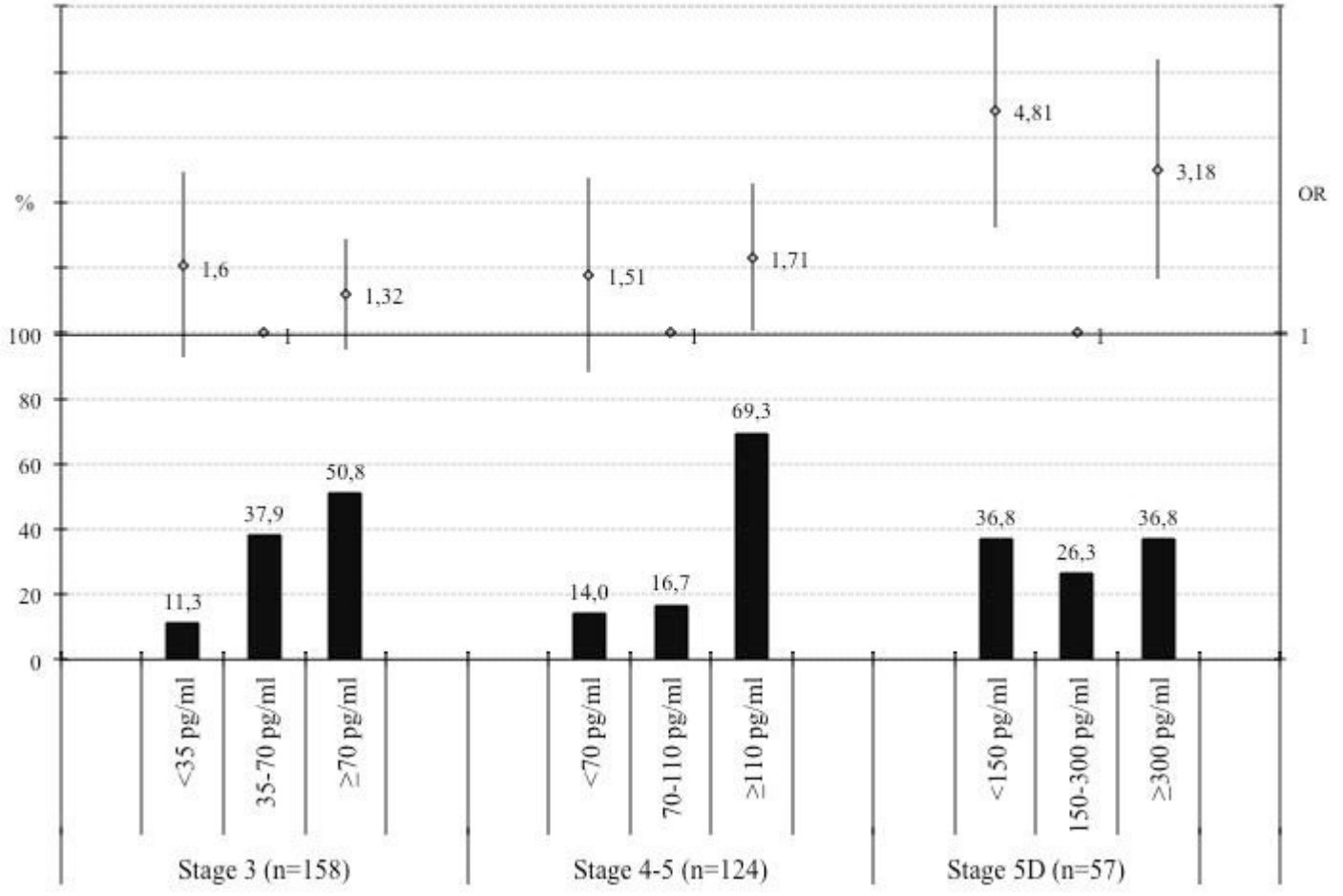

0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
CCIMT (mm) at baseline
0,035
0,03
0,025
0,02
0,015
0,01
0,005
0
-0,005
-0,01
Median Change in CCIMT (mm/y)
*
*
*
Stage 3
Stage 4-5
Stage 5D

Figure 2

Figure 3


%
OR
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69,3
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35-70 pg/ml
≥70 pg/ml
<70 pg/ml
70-110 pg/ml
≥110 pg/ml
<150 pg/ml
150-300 pg/ml
≥300 pg/ml
Stage 3 (n=158)
Stage 4-5 (n=124)
Stage 5D (n=57)